

APR 10 1930
682.13
A05
Number 90

Price 25 cents

REPRINT AND CIRCULAR SERIES
OF THE
NATIONAL RESEARCH
COUNCIL

ENZYME CATALYSTS

Seventh Report of the Committee on Contact Catalysis
National Research Council

E. F. ARMSTRONG and T. P. HILDITCH

Reprinted from the *Journal of Physical Chemistry*
Vol. XXXIII, 1929, pages 1441-1455.

Number 90

Price 25 cents

REPRINT AND CIRCULAR SERIES
OF THE
NATIONAL RESEARCH
COUNCIL

ENZYME CATALYSTS

Seventh Report of the Committee on Contact Catalysis
National Research Council

E. F. ARMSTRONG and T. P. HILDITCH

Reprinted from the *Journal of Physical Chemistry*
Vol. XXXIII, 1929, pages 1441-1455.

SEVENTH REPORT OF THE COMMITTEE ON CONTACT
CATALYSIS*
ENZYME CATALYSTS

BY E. F. ARMSTRONG AND T. P. HILDITCH

It has been the practice in these reports, whilst indicating the general progress in the knowledge of contact catalysis, to stress certain aspects of the subject in particular. For many of us, the ultimate aim of our strivings to understand the phenomena of nature is to gain some clearer understanding of the facts of life as portrayed by the chemical actions in the living cell. It has long been appreciated that, in such, catalytic agents play a leading, if not all-important, part and it is proposed therefore in this report to take stock of our knowledge of the catalysts of the cell.

The parallelism between inorganic catalysts and those involved in metabolic processes has been the subject of remark for rather more than a century. At times it has been held by some that the two divisions of chemical action have little or nothing in common beyond certain superficial resemblances; others have maintained the opposite view, and believed that fermentation and inorganic catalysis were varying instances of the same mode of operation of chemical activity. We are concerned at the moment rather to examine in some little detail various aspects of either class of phenomena in order to define with some clarity points of resemblance or of dissimilarity.

From the point of view of the chemist, vital changes are a series of balanced actions, generally reversible, following one after the other, which are only very crudely expressed by the chemical equation indicating the initial and final products of the reaction. Few, other than those who are really wedded to this field, realise how very delicate this balance is and how elaborate is the mechanism which is controlled apparently by those subtle agents for which the general name "enzymes" is used. These, the catalytic agents of protoplasm, determine the sequence and the magnitude of the molecular changes which distinguish living from dead matter and which include the biological synthesis of compounds, complicated or relatively simple, from carbon dioxide, water and nitrogen, and also actions of decomposition, for example, hydrolysis, oxidation (combustion) or reduction.

Apart from the complexity of the changes which proceed in the living cell, the fact that the study of these actions has been prosecuted most often from the point of view of physiology or pathology has involved a viewpoint

* Report of the Committee on Contact Catalysis of the Division of Chemistry and Chemical Technology of the National Research Council. Written by E. F. Armstrong and T. P. Hilditch, assisted by the other members of the committee: Messrs. H. A. Adkins, O. W. Brown, R. E. Burk, C. G. Fink, J. C. W. Frazer, J. N. Pearce, E. E. Reid, H. S. Taylor, and W. D. Bancroft, Chairman.

distinctly different from that of the student of the more conventional catalytic actions. In consequence it is not too easy to detect, in the extensive literature on enzyme action, information which is pertinent to the general question of the behavior and function of the actual catalyst. Re-direction of research on enzyme action in the sense indicated, and in the light of modern work on contact actions at the surface of inorganic catalysts, is important not only with reference to the development of biochemistry, but also in its possibilities as regards more frequent utilisation of enzymes in the field of applied organic chemistry. The recent advances in the biochemical production of butyl alcohol, acetone, glycerol, aldehyde, and lactic acid can undoubtedly be supplemented by those of other compounds; as is well known, there is scope for improvement and modernisation in the bacterial production of butyric and of citric acids, both of which are in increasing demand.

Our exact knowledge of the factors which affect the activity of enzyme catalysts is barely 25 years old. Since the broad character of these reactions and their general simplicity was first established, many papers have been published, to a large extent without reference to and apparently without knowledge of the earlier work, which have done much to obscure the subject.

Without going into detail, it can be concisely stated that the factors controlling the rate of enzyme action¹ are:—

1. The amount of the catalytic agent.
2. The concentration of the substrate upon which it acts.
3. The presence of substances which reduce the effective surface of the catalyst.
4. The influence of the products of change as depressants of a similar nature.
5. The acidity or hydrogen-ion concentration of the medium in which the amphoteric enzymes act.
6. Dehydrating agents affecting the inhibition and water relations of the catalyst.

It has been shown that the shape of the time curve expressing the hydrolysis of cane-sugar by invertase is ideally linear,² and that alterations in the rate of change leading to less simple relationships (frequently of a "logarithmic" character) are associated with subsidiary effects involving change in the effective activity of the enzyme. Association of one or more of the products of change with the enzyme is a common occurrence affecting the rate of action.

The amount of enzyme and substrate in combination at any one moment has been defined as the active mass. This conception has enabled a simple explanation of the time/change curves of enzyme activity to be given, including both the initial linear period, the subsequent logarithmic period and the final stages in which the rate of change is still slower. It cannot be too strongly emphasised that all more complicated mathematical equations to express the velocity constant throughout an enzyme change are unnecessary and fallacious.

The general features of the rate of change have led to the view that enzyme action takes place entirely at the surfaces of colloid particles suspended in the solution of the substrate and not between substances which are all in true solution.

Before discussing further the progress of a typical enzyme action and the susceptibility of the enzymic catalyst to alteration in activity by reason of the causes enumerated above, we may pass on to consider the evidence at present available as to the actual nature of enzymes. The statement that these act in the form of colloidal particles recalls the converse attitude taken by Bredig,³ who termed the dispersions or sols which he prepared from platinum and similar metals "inorganic ferments," on account of their similar behaviour to the "catalase" group of enzymes in effecting chemical actions such as the decomposition of hydrogen peroxide. The resemblances between a metallic sol and an enzyme are, however, of a somewhat superficial nature. Apart from the great complexity of chemical structure and the marked specificity in action which are the outstanding marks of the natural enzymes, the rate of action of Bredig's colloidal platinum is not of the same order as those of most of the usual preparations of enzymes, and falls far short of those of the relatively concentrated enzyme fractions prepared by Willstätter and his colleagues during their well-known recent investigations.

Since the most highly active fractions obtained are stated by these workers to have been still far from 100 per cent. enzyme, it follows that the metallic sols cannot be compared in any quantitative degree with the enzymes. Yet, from a qualitative point of view, the two classes of catalysts, are similar not only in certain aspects of chemical behaviour but also with reference to their temperature relationships. Ernst⁴ showed that colloidal platinum sols are closely parallel to the enzymes in this respect, possessing a comparatively low optimum temperature of more or less sustained activity, whilst showing greater initial, but more fugitive, activity at temperatures above the optimum; until a point is reached at which the catalytic power is suppressed completely. These phenomena are to be connected in both cases with changes effected by temperature in the colloidal surfaces, which are ultimately "coagulated" or altered so that the power of catalysing chemical actions disappears.

Whilst we may suppose, therefore, that so far as physical condition goes the enzymes are comparable most closely to metallic sols in the realm of inorganic catalysts, it is well to bear in mind that other analogies subsist between them and the more closely studied class of solid surface catalysts, notably the metals and metallic oxides.

In the first place, the similarity in regard to temperature effects is still present, the outstanding differences being the generally wider range of temperature over which the activity of a solid contact agent is exhibited and the fact that the working range of temperature of the latter is usually much higher than in the case of the natural enzymes. Finely-divided nickel, for instance, commences to show catalytic power below 100°C., its optimum

range in hydrogenation is usually about 140–180°C., and above about 350°C. its activity (in the absence, of course, of special “supports”) is soon suppressed.

Further, the so-called “promoter” or stimulant action of many substances on solid contact agents, and more especially the behaviour of mixed catalysts, are matters which it is interesting to note for comparison with the behaviour of enzymes. Readers of these reports will be well aware that the precise mechanism of catalyst stimulants and mixed catalysts is as yet far from perfectly understood. Willstätter, in his Faraday Memorial Lecture,⁵ refers to the possibility that a mixture of two components, whether by deformation of electron orbits or alteration of “affinity fields,” may result in a new catalyst having an “enzyme-like specificity and potency comparable with that of homogeneous compounds,” and proceeds to a possible analogy between such mixed catalysts and many enzyme systems which are known to be composed of more than one enzyme and to act differently from any one of the individual enzymes present.

It is well to remember, however, in this connection that there are two well-defined classes of mixed solid contact agents; in one case, an added component serves to increase the activity or potency of the original catalyst for effecting one and the same chemical change, whilst in another category we find a mixture of catalysts* which may induce a decomposition which either component alone fails to bring about (the action of “Hopcalite”⁶ in the oxidation of carbon monoxide at low temperatures is a good example of this class). The first-mentioned type, in which the specific activity of the original catalyst is merely accentuated by the presence of the added material, is much the more common, and there is considerable evidence (for example, the stimulating action of alumina or magnesia on nickel in hydrogenation)⁷ for the view that here the cause of the enhanced activity lies in something akin to more profound subdivision (i.e., greater effective surface) of the actual catalyst particles.

Again, the enzymes are well-known to be essentially specific in their action, a fact which indicates some close connection in molecular structure between them and the substrate on which they act. There is some little danger that the stress laid on the specificity of enzymes may be misleading if it is not carefully defined. Enzymes differ widely in the specific nature of their action. Some of the varieties of lipase, for example, are capable of hydrolysing a comparatively wide range of compounds belonging to the class of esters, and in particular they appear to act more or less indiscriminately on the fats irrespective of the particular nature of the fatty acids present. On the other hand, maltase, emulsin and most of the proteoclastic enzymes are far more specific, since most frequently they are only able to effect change in compounds of very definite stereochemical configuration, and it is in this respect that they are most differentiated from the solid contact agents.

* Professor Frazer of the Johns Hopkins University reports that he has succeeded in purifying certain of the oxide catalysts to such a degree that they are quite as active as, if not more active than, the mixture of oxide catalysts known as Hopcalite.

Equally, it must be borne in mind that inorganic (non-enzymic) catalysts are also highly specific in that the course of the action depends on the catalyst employed; enzymes share this property, but also, in many cases, possess the additional property of only being active with respect to certain members of a given class of compounds whose configuration, it may therefore be assumed, is compatible with that of the enzyme.

In the case of certain enzymes, substances which have similar structure to the substrate act to retard change though they cannot be hydrolysed by the enzyme—for example, the hydrolysis of the sugar maltose and of α -glucosides by the enzyme maltase of yeast is retarded in presence of glucose or of α -glucosides.⁸ This is in itself further proof that some form of combination of a chemical rather than a physical nature takes place between enzyme and substrate prior to hydrolysis. This corresponds with the addition-compound theory of catalysis which has found acceptance in connection with the action of nickel and other metallic catalysts.

To avoid any confusion of thought as to the specificity which characterises solid contact agents, attention may be drawn to a few very obvious cases. Probably the most useful example is the old one of the varying results obtained when alcohol vapour is exposed to the action, on the one hand, of nickel, copper or a similar metal, and, on the other, of oxides such as alumina or theoria.⁹ The two groups of catalysts exert specific influences respectively of dehydrogenation or dehydration. Furthermore, Sabatier's classical work on the catalytic behaviour of a whole range of metallic oxides¹⁰ will be recalled, wherein the specific action produced varies, according to the particular oxide, over the range exclusive dehydration—mixed dehydration and dehydrogenation—exclusive dehydrogenation.

Another familiar instance is the varying action on a mixture of carbon monoxide and hydrogen of different catalytic compounds: nickel, palladium, or platinum cause the production of methane¹¹ (with carbon dioxide under certain conditions), zinc oxide or mixed zinc-chromium oxides¹² yield methyl alcohol almost exclusively, and iron in presence of alkaline oxides¹³ furnishes a mixture of hydrocarbons, alcohols and other products.

It is possible to point to other actions of solid catalysts which, although not comparable with the stereochemical selectivity of the enzymes, are of a still more specific nature than those just cited. For example, hydrogenation of an isolated ethylenic linkage in a normal aliphatic chain (as in oleic acid) is accompanied by the production of some of the geometrically-isomeric form of the original ethylenic derivative, together with that of isomeric compounds containing the ethenoid or double bond in positions adjacent to that originally occupied;¹⁴ and it is reported that palladium (and, possibly, copper) yields higher proportions of the isomeric forms than does nickel when employed as catalyst. The studies of Adkins¹⁵ and his co-workers on the different types of catalytic change induced by alumina prepared in different ways is another instance of pronounced selectivity in the action of an inorganic catalyst. Again, Willstätter¹⁶ has stated that hydrogenation of naphthalene in presence of platinum yields exclusively *cis*-decahydronaphthalene,

whilst with nickel the chief product is the *trans*-compound; and similar results have been reported in connection with the methylcyclohexanols and menthols produced in the hydrogenation of cresols and thymol. Most striking of all, perhaps, is the phenomenon of selective hydrogenation,¹⁷ whereby, in some cases, one of a number of ethylenic linkages is hydrogenated preferentially, to the almost complete exclusion of the rest; or, in other cases, hydrogenation of, for example, a diolefinic compound proceeds in such a way that mono-olefinic derivatives are formed before any notable amount of saturated substance appears, although either of the original double bonds may be and are attacked (leading to a mixture of mono-ethylenic isomerides). The work of S. Lebedev¹⁸ and his colleagues has shown especially well the conditions which determine selective action of this kind in some cases; and stress has been laid by these investigators upon the great influence on the course taken by the hydrogenation of the presence of substituent alkyl groups.

The examples quoted of variation in the proportion of geometrical isomerides formed during hydrogenation and of the influence of substituent alkyl groups (i.e., branched-chain configuration) on the course of selective hydrogenation will be admitted to approach somewhat towards the still more delicate stereochemical relationships which are apparent between some enzymic catalysts and their substrates.

Consequently, when Willstätter, in his Faraday Memorial Lecture which has already been mentioned, says "there appears to be a deep gulf between the inorganic catalysts and the enzymes" (adding the further statement "In reality this gulf has already been bridged by accurately-defined organic compounds which act like enzymes"), it would appear that something less than justice is done to the analogies already existing between the two classes of catalyst. From a general standpoint, almost all the chemical transformations effected by enzymes are capable of being paralleled, in greater or smaller measure, by analogous transformations which are realised by one or other of the inorganic catalysts.

We can go somewhat further than this, however, if we consider the few cases of "accurately-defined organic compounds" which have been investigated from the point of view of catalytic action. In the first place, Willstätter has demonstrated that some of the natural complex products of or interveners in vegetable and animal metabolism possess catalytic properties somewhat similar to enzymes, although they are by no means enzymes in the usual sense of the term. Chlorophyll, according to Willstätter and Stoll,¹⁹ may build up carbohydrates from carbon dioxide by rearrangement of the latter (under the influence of radiation) in the form of the labile chlorophyll-carbon dioxide complex. Willstätter and Pollinger²⁰ have found that oxyhaemoglobins from different animal species behave like feeble peroxydase, and vary in activity according to their source. Kuhn and Brann²¹ have established further that, when haemoglobin is resolved into its components, the resultant haemin has no peroxydase properties, but shows distinct catalase activity. This is almost direct evidence that the specific action of organic-compound catalysts depends on the chemical constitution of the latter.

Another line of argument may be based on the well-known synthetic agents for the hydrolysis of fats which were developed originally by Twitchell,²² who condensed benzene or naphthalene with oleic acid by means of sulphuric acid and obtained a product containing one or more free sulphonic acid groups united to the oleic acid by means of the aromatic nucleus. Many variants of the original agent have been proposed, and latterly sulphonated petroleum hydrocarbons of certain types have been suggested. All these contact agents have in common the presence of a strongly hydrophilic sulphonic acid group united with a hydrocarbon or fatty acid residue of high molecular weight. Consequently they may be supposed to exert marked affinity both for fats, fatty acids and water, whilst the actual hydrolysis is conditioned by the presence of the highly active sulphonic acid radical present in the complex. The Twitchell agent is, in fact, probably the nearest synthetic approach which has been made to the production of an artificial compound with properties and structure resembling in essentials those of a natural enzyme.

Summing up all these analogies and inferences, it seems that there is good ground to come to the following conclusions:—

1. The enzymes exert hydrolytic action in virtue of the presence of definite chemical groupings adapted to form intermediate complexes with the substrate and water or other interactant.
2. They owe their specific powers (especially those connected with stereo-isomeric conditions) in all probability to their own asymmetric configuration as natural synthetic products.
3. Many general analogies can be traced between the recognized behaviour of inorganic contact agents (especially those operating on liquid media) and that of enzymes.
4. From a physical point of view, they are most nearly similar in general properties to the dispersed sol forms of inorganic catalysts; but the existing evidence goes to show fairly conclusively that their superior activity and delicacy of action cannot be accounted for simply by differences in the degree of dispersion.

All this of course is only one side of the possible nature of an enzyme. We have still to consider their formation in the cell and the development of their catalytic powers, together with other phenomena characteristic of enzymes. Amongst the latter, the influences exerted by "co-enzymes" and "anti-enzymes" are respectively analogous to the inorganic catalyst promoters and poisons. Substances toxic to either group of catalysts probably act in a similar manner, the primary cause being in both cases selective adsorption of the toxic compound by the catalyst with resulting exclusion of most or all of the material to be acted upon from the catalytically active centres. With enzymes, however, it usually happens that some of the products of change are themselves prone to enter into association or be strongly adsorbed by the enzyme, and consequently the apparent activity of the latter frequently declines as the products of change accumulate in the system. This state of affairs has rarely been encountered so far in the case

of most actions at the surface of solid contact agents, but it has been met with in several common instances of homogeneous catalysis: for example, in some cases of chlorinations of liquid organic compounds in presence of "carriers," and in the Friedel-Crafts synthesis wherein, as shown by Steele,²³ the aluminium chloride forms more or less definite complexes with the original hydrocarbon, the organic halide, and the hydrocarbon produced in the interaction.

The part played by "co-enzymes" is not quite so closely parallel to the ordinary case of promoter action in inorganic catalysts. It is, indeed, by no means agreed as to what the precise function of a "co-enzyme" is, but the main distinction between this type of substance and a catalyst promoter is that, whilst the latter is an aid to the development of more potency, the "co-enzymes" are indispensable for the progress of many enzyme actions. The mechanism by which co-enzymes act is little understood at present. The presence of a suitable electrolyte in appropriate concentration seems to be the chief factor in some cases, but in others the mere presence of a simple salt is not sufficient; thus zymase is not restored to activity by the simple presence of a phosphate solution, although phosphates are necessary. There is reason to suppose, with Harden,²⁴ that the real co-enzyme is in this case a hexose-phosphoric-acid ester. It is further possible that, in some cases, the "co-enzyme" is a substance which activates or releases the enzyme itself from a still more complex pre-existent compound in the cell.

As is well-known, the activity of enzymes is peculiarly susceptible to the acidity or hydrogen-ion concentration of the medium in which they act. This is one of the most prominent distinctions between enzymes and inorganic catalysts, but it may be remarked that, even in this respect, analogies are not lacking between the two classes. The action of nickel as a hydrogenating catalyst, for example, has been shown to be greatly influenced by the general reaction of the compound hydrogenated; this is shown most clearly, perhaps, in the increased ease of hydrogenation of phenols in presence of a small, optimum concentration of sodium carbonate, and again, whilst it is generally recognised that nickel hydrogenates acidic compounds with relative difficulty, it is also probable that basic compounds are less susceptible than neutral compounds to hydrogenation in presence of nickel. At all events, there is some reason to believe that ammonia acts as a catalyst poison towards nickel, and basic ring-systems such as pyridine, pyrrol or quinoline are notoriously difficult to hydrogenate by comparison with benzene or naphthalene. Another example of acidity controlling rate of action is due to the recent work of Conant and Brammann,²⁵ who have observed variations of a million-fold in the rate of acetylation of naphthol by acetic anhydride as a result of varying the acetic acidity of the reaction medium.

The exact bearing of the optimum hydrogen-ion concentration on the activity of an enzyme has yet to be understood. Nearly all enzymes are very sensitive to slight changes in the acidity or alkalinity of the solution and are most active only at a definite hydrogen-ion concentration; the most effective value of the latter varies according to the enzyme, the usual range

being from media slightly more acid to media slightly more alkaline than pure water. Enzymes are, as a rule, of an amphoteric nature and doubtless this has considerable relation to their activity over only a limited range of acidity and their maximum activity at a definite hydrogen-ion concentration. As colloids they are susceptible to heat and their structure is impaired or destroyed at moderate temperatures.

The work of Willstätter and his school on the attempted isolation of various enzymes has afforded definite results of an important character, but no enzyme has so far been produced in a condition approaching purity. The work has been notable for the degree of utility manifested in successive processes of adsorption and elution carried out with carefully selected agents. It has been proved practicable in this way to separate enzymes from colloidal substances not closely related to them, though beyond this, except by very special procedure, it has not been found possible to go. The ramifications of this research demand that the original papers²⁶ should be studied by those interested in the details of the procedure. Here it must suffice to cite the separation of the enzymes of pancreas as an instance. If the mixture is treated with the stable gel form of aluminium hydroxide ("γ-alumina"), lipase present is adsorbed and can be removed by elution of the alumina with a solution of feebly alkaline phosphate; amylase and trypsin remain, and the latter is selectively adsorbed by kaolin.

In the same way, it has been shown that invertase can be almost completely freed from carbohydrates, proteins and phosphorus compounds, with an accompanying increase in activity of the order of over three thousandfold. Proteins are also believed to form no essential part of the lipoclastic enzymes and the peroxydases.

An especially interesting case is that of the vegetable peroxydases, which in the hands of Willstätter and Stoll²⁷ yielded a concentrate containing a good deal of carbohydrate (sugars) and a definite iron content of about 0.5%. Further purification by selective adsorption led to a still more active preparation of the enzyme, but the sugar constituents disappeared in the process and the iron content diminished to 0.06%. Thus, whilst it appears certain that the carbohydrates present were merely accompaniments to the enzyme itself, it is also doubtful how far the presence of combined iron is necessary. The interesting observation was made that, in the highest stages of concentration reached, the active material assumed a reddish-brown colour reminiscent of that of the porphyrins.

Keilin²⁸ has recently published a very important contribution on the respiratory processes of cells of aerobic organisms, vegetable and animal, and has given definite proof that the material responsible for at least one portion of the complicated respiratory mechanism of the cell is a widely distributed respiratory pigment, cytochrome, which is composed of three haematin compounds (a', b' and c') and an unbound haematin compound similar to the protohaematin of haemoglobin. These haematin compounds are responsible for the thermostable peroxydase action, which is shown by

the oxidation of various chromogens, such as benzdine, guaiacum, and *p*-phenylenediamine, in presence of hydrogen peroxide. Keilin's work seems to be conclusive evidence at last of the general nature of the peroxydase class of "enzymes," and indicates that "peroxydases" are identical with certain types of the complex organic compounds known as haematin—these "enzymes" at least may now be claimed to be complex carbon-compound catalysts. Whether the hydrolytic and other classes of enzymes will in course of time be proved to be equally definite entities remains to be seen. Quite possibly a difference may be revealed in that, whilst a specific haematin (peroxydase) may be capable of promoting oxidation processes with reference to a comparatively wide variety of compounds, a process such as hydrolysis of a particular compound may demand the presence not merely of a suitable hydrolytic mechanism but of such a mechanism so arranged in conjunction with the rest of a complex molecular system as to satisfy configurational or other intramolecular requirements.

Not the least interesting part of recent biological work is the differentiation made between certain features which are shown to be characteristic of the enzyme itself and other very characteristic features of enzyme action which are equally shown to be due to the influence of other compounds present with the enzyme. Thus the lipase of the human pancreas is most active in an alkaline medium, whilst that of human gastric juice is most active under feebly acidic conditions (pH 5–6); when the latter is submitted to the adsorbent action of kaolin, however, some compound (or compounds) is removed and the reaction of the enzyme is transferred to an optimum on the alkaline side at pH 8.²⁹ The optimum hydrogen-ion concentration for a given enzyme would thus seem to be dependent, not on the ultimate enzyme alone, but on the accompanying components of the complex mixture or system on which the term natural enzyme is at present conferred.

On the other hand, the specificity of any enzyme has so far been found to remain unchanged throughout any concentration process which has been applied and in spite of exceedingly great increases in the relative activity of the purified enzyme and the original material. This holds for examples of selective stereochemical action in the cases of invertase, maltase, etc. and of certain animal lipases which hydrolyse the *d*- and *l*-forms of certain optically-active esters at unequal rates;³⁰ and it may therefore be inferred, from the present experimental evidence, that the selective chemical and configurational action of enzymes is an essential property of the catalyst itself.

Another development has suggested that some enzymes (e.g., trypsin) only exhibit their full activity when released or stimulated by a specific type of complex associated with them (e.g., the kinases). Kinases were formerly supposed by Bayliss and Starling³¹ to be themselves enzymes which in the first place converted an inactive complex "trypsinogen" into the enzyme trypsin; according to Willstätter,³² trypsinogen is itself an enzyme of relatively feeble activity which, when associated with an activator or "co-enzyme" of the kinase group develops much greater potency. This explanation is based

on adsorption experiments whereby, employing casein precipitated *in situ* as adsorbent, the kinases can be removed, leaving all the trypsin in solution.

It is perhaps permissible to attempt to sum up the evidence presented by the data discussed in the preceding pages in the form of a hypothetical suggestion of what may be the essential parts of a natural enzyme *per se*.—

1. The physical form of the enzyme is that of a dispersed colloidal sol, the colloid molecule probably being of great complexity.

2. As regards the chemical nature of the enzyme molecule itself, it is evident that neither protein, carbohydrate, organic phosphorus nor iron compounds, etc., is necessarily an integral component. Yet it must be concluded that the whole of the molecular complex is unlikely to be involved in the enzymic changes effected in the appropriate substrates, and thus we are reduced to a somewhat vague picture of a large molecule of undefined composition which, however, contains certain groupings of highly specific character and, usually, configuration, which are able to enter into association with specific substrates.

3. At the same time, however, the whole molecule is peculiarly liable to form associated complexes with other large molecular species, and it should be further observed that the presumed reactive groupings must be, if not actually involved in these associations, considerably influenced thereby. For, not only the rate of action, but also the optimum character of the reaction medium, is largely affected by these apparently extraneous complications: only the specific nature of the chemical change effected by the enzyme itself appears to persist unaltered whatever the varying environment, so to speak, of the latter may be.

4. The question of enzyme stimulation or “co-enzyme” activity appears to be closely connected with the combinations with other colloidal molecules to which enzymes appear to be prone. Some instances of the “release” of an enzyme in the cell in the active state seem to be explicable on the theory of intervention or stimulation by association with a co-enzyme; in others this possibility does not seem entirely clear.

5. It appears on the whole that the catalytic properties of an enzyme are at present best interpreted by regarding it as an instance of a catalyst which, for practical purposes, is in a labile or dynamic condition. We do not mean by this that the essential catalyst, the enzyme *per se*, is continually made and destroyed or re-made. It is suggested, on the contrary, that the enzyme itself may well be synthesised once for all in the cell, but that, by its capacity for association or interaction with other products of the cell (whether enzyme stimulants or retarders, or comparatively inert), its activity is peculiarly liable to a variety of delicately-adjusted conditions and is very largely controlled by its environment at any given time.

The properties revealed by the foregoing survey of the present evidence are typical of provision in nature of means whereby a close control over chemical reactions is exercised. The very great activity of enzymes as com-

pared, for example, with mineral acids hardly requires emphasis. The enormous activity of some of Willstätter's partially concentrated enzymes makes possible some idea of that of a pure enzyme, freed or "released" from any of the accompanying complexes of similar chemical character but catalytically inert. The close combination of enzyme and related substrate in a large colloidal complex must set up conditions where a minimum amount of energy is required to bring about change.

In life the enzyme is not normally in a free state—it is only liberated as required. Either the water equilibrium of the cell is such that no simple water molecules are available to complete the hydrolytic circuit so that before action begins dilution must take place. This can be effected by the entry into the cell of a stimulant or hormone such as is controlled by the nervous system. Alternatively, the enzyme itself is not in the free state and must first be liberated from a complex colloid by another enzyme. A third possibility is the need of a co-enzyme, as for example the cozymase of cell-free alcoholic fermentation.

Thus far we have mainly focussed our attention on the nature of the enzymic catalyst and, to some extent, the general mechanism of its action. It remains to direct the notice, particularly of organic chemists, to the chemical changes involved in the course of synthesis and decompositions in the living cell. The lacunae in our chemical knowledge here are colossal. Investigations into such matters are admittedly most difficult; but the possibilities are extraordinarily inviting, and work such as that of Neuberg³³ and others on the alcoholic fermentation of glucose and fructose indicates that the problems set are not by any means completely insoluble.

Practically the only actions of enzyme chemistry which have been intensively studied are cases of simple hydrolysis or (conversely) withdrawal of the elements of water. The chemical changes whereby chains of carbon atoms are assembled or disrupted in nature have not yet been studied from the standpoint of organic chemistry, if we except the instances of possible stages whereby carbohydrates are built up from a formaldehyde complex, and the work, just mentioned, on the degradation of hexoses into alcohol, carbon dioxide, etc., by fermentation (together with recent related work by Langwell and others³⁴ which elucidates to some extent the possible transitions which take place when cellulose or sugars are submitted to the action of certain bacteria).

The manner in which sugars, and perhaps other materials, are built up into fatty acids, and the latter assembled into glycerides (neutral fats) of specific constitution; the evolution of the numerous types of protein-matter; the disruption or further transformation of the three main classes of natural organic compounds—carbohydrates, fats, proteins; the production of terpene (or isoprene) derivatives—all these await interpretation in terms of organic chemistry. All that is available at present in many cases is a facile "paper" explanation wherein methyl (or other alkyl) groups, hydroxyl radicals, etc., are lopped off at will, unsaturated centres are similarly transposed, eliminated or introduced, and, in general, a scheme of things is envisaged by

the biochemist which, if it were realisable in the laboratory or the factory, would open up joyous vistas to the technical synthetic organic chemist.

Definite information is badly needed on this side of enzyme chemistry. As already stated, the work is most difficult; the more so, since intermediate stages of the chemical changes in progress will doubtless often involve the production of organic compounds in unusually labile or unfamiliar forms. Thus the pyruvic acid, methylglyoxal, acetaldehyde and even formaldehyde of the laboratory are undoubtedly not identical, as regard physical and chemical properties, with the corresponding compounds which are transitorily produced during the low-energy transformations proceeding in a comparatively dilute aqueous medium in the living cell.

Doubtless this is getting rather remote from the direct problems of contact catalysis, yet it has a very definite bearing on the possibility of extending the use of enzymes in industrial processes. Two general methods are discernible in the latter connection.

The older one, which is obviously capable of much wider extension, is to select a culture of an organism which produces enzymes capable of inducing definite, and sometimes unusual, chemical actions, leading to the production of useful material. This procedure, long applied to the manufacture of vinegar, lactic, butyric and to some extent citric acids, is receiving fresh attention in the latter case and has of course made striking contributions to industrial chemistry in latter years in the lipase process for hydrolysis of fats³⁵ and, especially, in the modern method of manufacture of butyl alcohol and acetone.³⁶ It is, in effect, the production of new, or rather hitherto unnoticed, enzymes by the cultivation of special varieties of microflora, and, consequently, falls more within the province of the bacteriologist than the chemist.

The alternative method is to acquire sufficient knowledge of the chemical course taken by an enzyme process and to endeavour to modify its progress by carrying it out in the presence of added chemicals. Two familiar instances will exemplify this method of attack:—

1. The presence of sulphite or of a mild alkali during yeast fermentation of glucose or fructose causes the acetaldehyde (or acetaldehyde complex) transitorily produced to be respectively fixed as bisulphite compound or “dismuted” into alcohol and acetic acid (or polymerized); consequently the elements of hydrogen which would have transformed acetaldehyde into alcohol are utilised otherwise, and glycerol results—a process which has been, and very probably will again be, used on the large scale for the manufacture of glycerine.³⁷

2. The chemical course of the butyl alcohol-acetone fermentation has been studied and grounds for believing that butyric acid and acetic acid were intermediary products were obtained. It has further been shown that, within limits, butyl alcohol, acetone and some other alcohols or ketones can be bio-

chemically produced by adding to a fermenting mash (undergoing the *Granulobacter* fermentation) butyric, acetic or other appropriate acid, of course in such quantities that the acidity of the mash does not exceed a prescribed limit at any given time.³⁸

Evidently there are possibilities of considerable progress along these lines, and such progress will be the more rapid as the chemical course of enzyme actions receives increasing attention from organic chemists.

We do not go so far as to suggest that in all cases an enzyme process will readily compete with the more usual forms. The great advantage of low heat consumption in the actual process may be offset sometimes by the difficulty of maintaining an appropriate organism in existence, or by the dilution at which a particular process must necessarily be carried out, leading to excessive fuel charges in recovery of the products. Several instances in the past few years nevertheless give encouragement to the view that enzyme chemistry is only at the commencement of its technical utilisation.

The aspect of catalysis selected for this report has led to a somewhat more general and diffuse treatment than that given by our predecessors. We make no apology for this, however, but rather claim that we have endeavoured to give as careful a statement as the facts warrant of a somewhat elusive subject, expressed in the simplest possible language. If we have interested any workers on catalysis in the importance and potentialities of enzyme chemistry, considered primarily from the point of view of catalytic action, and if this report attracts fresh investigators to this field of contact catalysis, we shall have attained our end.

Bibliography

- ¹ Croft Hill: J. Chem. Soc., 73, 634 (1898); 83, 578 (1903); V. Henri: Z. physik. Chem., 39, 194 (1901); 51, 19 (1905); E. F. Armstrong: J. Chem. Soc., 83, 1305 (1903); Proc. Roy. Soc., 73B, 500, 516 (1904); 74B, 188, 195 (1904); 76B, 592 (1905); H. E. and E. F. Armstrong: Proc. Roy. Soc., 79B, 365 (1907); 86B, 561 (1913); H. E. and E. F. Armstrong and E. Horton: Proc. Roy. Soc., 80B, 321 (1908); 85B, 359 (1912); 86B, 262 (1913); H. E. Armstrong and W. H. Glover: Proc. Roy. Soc., 80B, 312 (1908); H. E. Armstrong and E. Horton: Proc. Roy. Soc., 82B, 349 (1910); H. E. Armstrong, M. S. Benjamin and E. Horton: Proc. Roy. Soc., 86B, 328 (1913); H. E. Armstrong and H. W. Gosney: Proc. Roy. Soc., 88B, 176 (1914); H. von Euler: Z. physiol. Chem., 85, 192 (1913); Biochem. Z., 84, 402 (1917); 85, 406 (1918); Z. Elektrochemie, 24, 173 (1918); Trans. Faraday Soc., 24, 651 (1928); and many other papers.
- ² E. F. Armstrong: Proc. Roy. Soc., 73B, 500 (1904).
- ³ G. Bredig: Z. physik. Chem., 31, 258 (1899).
- ⁴ C. Ernst: Z. physik. Chem., 37, 448 (1901).
- ⁵ R. Willstätter: J. Chem. Soc., 1927, 1359.
- ⁶ A. B. Lamb, W. C. Bray and J. C. W. Frazer: J. Ind. Eng. Chem., 12, 217 (1920); W. A. Whitesell and J. C. W. Frazer: J. Am. Chem. Soc., 45, 2848 (1923).
- ⁷ E. F. Armstrong and T. P. Hilditch: Proc. Roy. Soc., 103A, 586 (1923).
- ⁸ E. F. Armstrong: Proc. Roy. Soc., 73B, 316 (1904).
- ⁹ P. Sabatier: "La catalyse en chimie organique," 48 (1920).
- ¹⁰ P. Sabatier and A. Mailhe: Ann. Chim. Phys., (8) 20, 289 (1910).
- ¹¹ P. Sabatier and J. B. Senderens: Compt. rend., 134, 514 (1902); E. F. Armstrong and T. P. Hilditch: Proc. Roy. Soc., 103A, 25 (1923).
- ¹² G. Patart: Compt. rend., 179, 1330 (1924); Chim. et Ind., 13, 179 (1925); E. Audibert: Chim. et Ind., 13, 186 (1925); 20, 1015 (1928); H. S. Taylor and C. B. Kistiakowsky: J. Am. Chem. Soc., 49, 2468 (1927).

- ¹³ F. Fischer and H. Tropsch: *Brennstoff. Chem.*, **4**, 276 (1923); **5**, 201 (1924); **7**, 97, 299 (1926); **8**, 165 (1927); *Ber.*, **59**, 830 (1926).
- ¹⁴ T. P. Hilditch and N. L. Vidyarthi: *Proc. Roy. Soc.*, **122A**, 552 (1929).
- ¹⁵ H. Adkins and co-workers: *J. Am. Chem. Soc.*, **44**, 385, 2175 (1922); **47**, 807 (1925).
- ¹⁶ R. Willstätter and F. Seitz: *Ber.*, **57B**, 683 (1924).
- ¹⁷ E. F. Armstrong and T. P. Hilditch: *Proc. Roy. Soc.*, **108A**, 121 (1925).
- ¹⁸ S. V. Lebedev, G. G. Kobliansky and A. O. Yakubchik: *J. Chem. Soc.*, **127**, 417 (1925).
- ¹⁹ R. Willstätter and A. Stoll: *Ber.*, **50**, 1791 (1917).
- ²⁰ R. Willstätter and A. Pollinger: *Z. physiol. Chem.*, **130**, 281 (1923).
- ²¹ R. Kuhn and L. Brann: *Ber.*, **59B**, 2370 (1926).
- ²² E. Twitchell: *J. Am. Chem. Soc.*, **22**, 22 (1900).
- ²³ B. D. Steele: *J. Chem. Soc.*, **83**, 1470 (1903).
- ²⁴ A. Harden and W. J. Young: *Proc. Roy. Soc.*, **77B**, 405 (1906); **78B**, 369 (1906); **80B**, 299 (1908); **81**, 336 (1909); **82**, 321 (1910).
- ²⁵ J. B. Conant and G. M. Bramman: *J. Am. Chem. Soc.*, **50**, 2305 (1928).
- ²⁶ R. Willstätter and co-workers: *Ann.*, **422**, 47 (1921); **425**, 1 (1921); **427**, 111 (1921); **430**, 269 (1923); *Ber.*, **55**, 3601 (1922); *Z. physiol. Chem.*, **123**, 1, 181 (1922); **125**, 132 (1923); etc.
- ²⁷ R. Willstätter and A. Stoll: *Ann.*, **416**, 21 (1918); **430**, 269 (1923).
- ²⁸ D. Keilin: *Proc. Roy. Soc.*, **104B**, 206 (1929).
- ²⁹ R. Willstätter and F. Memmen: *Z. physiol. Chem.*, **133**, 247 (1924).
- ³⁰ H. D. Dakin: *J. Physiol.*, **30**, 253 (1903); **32**, 199 (1905); R. Willstätter and F. Memmen: *Z. physiol. Chem.*, **138**, 216 (1924); R. Willstätter and H. Kumagawa: *Z. physiol. Chem.*, **146**, 151 (1925); etc.
- ³¹ W. M. Bayliss and E. H. Starling: *J. Physiol.*, **32**, 129 (1905).
- ³² R. Willstätter and E. Waldschmidt-Leitz: *Z. physiol. Chem.*, **132**, 181 (1924); **142**, 217 (1925).
- ³³ C. Neuberg and J. Kerb: *Biochem. Z.*, **58**, 158 (1913); C. Neuberg and J. Hirsch: *Biochem. Z.*, **100**, 304 (1919); C. Neuberg and E. Reinfurth: *Ber.*, **52B**, 1677 (1919); *Biochem. Z.*, **106**, 281 (1920); C. Neuberg, F. F. Nord and E. Wolff: *Biochem. Z.*, **112**, 144 (1920).
- ³⁴ F. Nathan: *J. Soc. Chem. Ind.*, **42**, 279T (1923); A. H. Lymn and H. Langwell: *J. Soc. Chem. Ind.*, **42**, 280T (1923); A. C. Thaysen: *J. Soc. Chem. Ind.*, **42**, 283T (1923); C. J. J. Fox and L. Hall: *J. Soc. Chem. Ind.*, **46**, 281T (1927).
- ³⁵ Y. Tanaka: *J. Chem. Ind. Tokio*, **21**, 112 (1918).
- ³⁶ A. C. Thaysen and co-workers: *J. Inst. Brewing*, **27**, 529 (1921); **33**, 236 (1927); A. Gill: *J. Soc. Chem. Ind.*, **38**, 273T (1919); H. B. Speakman: *J. Soc. Chem. Ind.*, **38**, 155T (1919); *J. Ind. Eng. Chem.*, **12**, 581 (1920); *J. Biol. Chem.*, **41**, 319 (1920); **43**, 401 (1920).
- ³⁷ W. Connstein and K. Ludecke: *Wochenschrift für Brauerei*, **1919**, May 10; *Ber.*, **52**, B, 1385 (1919); J. R. Eoff, W. V. Linder and G. F. Beyer; *J. Ind. Eng. Chem.*, **11**, 842, 845 (1919).
- ³⁸ J. Reilly, W. J. Hickinbottom, F. R. Henley and A. C. Thaysen: *Biochem. J.*, **14**, 228, (1920).

COMMITTEE ON CONTACT CATALYSIS

First Report. By Wilder D. Bancroft, Chairman. In Collaboration with the other members of the Committee. (Reprint No. 30. April-July, 1922. Pages 43. Price \$0.50.)

Second Report. By Wilder D. Bancroft, Chairman. In collaboration with the other members of the Committee. (Reprint No. 50. December, 1923. Pages 141. Price \$0.50.)

Third Report. By Hugh S. Taylor. In collaboration with the other members of the Committee. (Reprint No. 59. September, 1924. Pages 46. Price \$0.50.)

Fourth Report. By Hugh S. Taylor. In collaboration with the other members of the Committee. (Reprint No. 66. February, 1925. Pages 27. Price \$0.50.)

Fifth Report. By Emmet Reid. In collaboration with the other members of the Committee. (Reprint No. 78. July, 1927. Pages 31. Price \$0.50.)

Sixth Report. By Robert E. Burk. In collaboration with the other members of the Committee. (Reprint No. 83. November, 1928. Pages 45. Price \$0.50.)

Seventh Report. Enzyme Catalysts. By E. F. Armstrong and T. P. Hilditch. (Reprint No. 90. October, 1929. Pages 15. Price \$0.25.)

Orders, accompanied by remittance,
should be addressed to

Publication Office
NATIONAL RESEARCH COUNCIL
WASHINGTON, D. C.